

## DMSO-Free Programmed Cryopreservation of Fully Dissociated and Adherent Human Induced Pluripotent Stem Cells.

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**Authors:** Igor I Katkov, Natalia G Kan, Flavio Cimadamore, Brandon Nelson, Evan Y Snyder, Alexey V Tersikh

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### Public Summary:

THREE MODES FOR CRYOPRESERVATION (CP) OF HUMAN IPSC CELLS HAVE BEEN COMPARED: STD: standard CP of small clumps with 10% of CPA in cryovials, ACC: dissociation of the cells with Accutase and freezing in cryovials, and PLT: programmed freezing of adherent cells in plastic multiwell dishes in a programmable freezer using one- and multistep cooling protocols. Four CPAs were tested: dimethyl sulfoxide (DMSO), ethylene glycol (EG), propylene glycol (PG), and glycerol (GLY). The cells in ACC and PLT were frozen and recovered after thawing in the presence of a ROCK inhibitor Y-27632 (RI). EG was less toxic w/o CP cryopreservation than DMSO and allowed much better maintenance of pluripotency after CP than PG or GLY. The cells were cryopreserved very efficiently as adherent cultures (+RI) in plates (5-6-fold higher than STD) using EG and a 6-step freezing protocol. Recovery under these conditions is comparable or even higher than ACC+RI. Conclusions. Maintenance of cell-substratum adherence is a favorable environment that mitigates freezing and thawing stresses (ComfortFreeze((R)) concept developed by CELLTRONIX). CP of cells directly in plates in ready-to-go after thawing format for HT/HC screening can be beneficial in many SC-related scientific and commercial applications such as drug discovery and toxicity tests.

### Scientific Abstract:

THREE MODES FOR CRYOPRESERVATION (CP) OF HUMAN IPSC CELLS HAVE BEEN COMPARED: STD: standard CP of small clumps with 10% of CPA in cryovials, ACC: dissociation of the cells with Accutase and freezing in cryovials, and PLT: programmed freezing of adherent cells in plastic multiwell dishes in a programmable freezer using one- and multistep cooling protocols. Four CPAs were tested: dimethyl sulfoxide (DMSO), ethylene glycol (EG), propylene glycol (PG), and glycerol (GLY). The cells in ACC and PLT were frozen and recovered after thawing in the presence of a ROCK inhibitor Y-27632 (RI). EG was less toxic w/o CP cryopreservation than DMSO and allowed much better maintenance of pluripotency after CP than PG or GLY. The cells were cryopreserved very efficiently as adherent cultures (+RI) in plates (5-6-fold higher than STD) using EG and a 6-step freezing protocol. Recovery under these conditions is comparable or even higher than ACC+RI. Conclusions. Maintenance of cell-substratum adherence is a favorable environment that mitigates freezing and thawing stresses (ComfortFreeze((R)) concept developed by CELLTRONIX). CP of cells directly in plates in ready-to-go after thawing format for HT/HC screening can be beneficial in many SC-related scientific and commercial applications such as drug discovery and toxicity tests.

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